Supplemental Document

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Illumination angle correction during image acquisition in light-sheet fluorescence microscopy using deep learning: supplement

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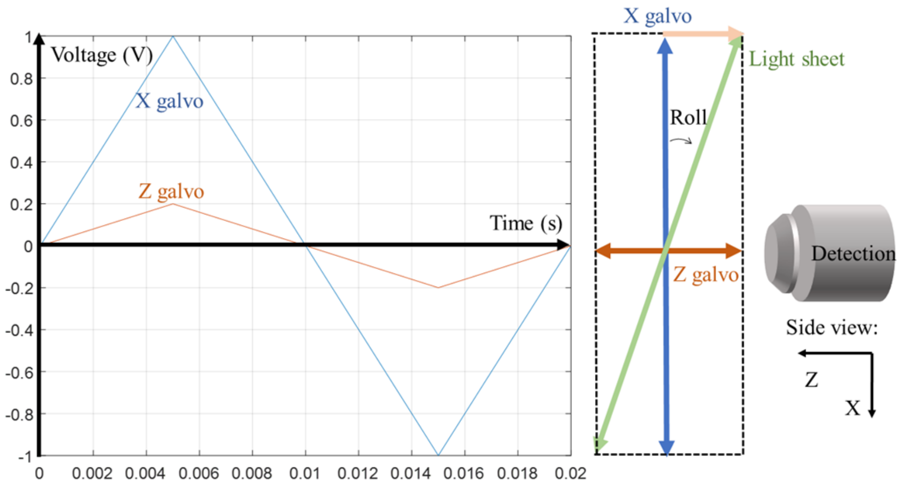

Fig. S1. The voltage diagram that is used to control the light-sheet roll angle. (Left) Voltage diagram for one period. (Right) The resulting roll angle.

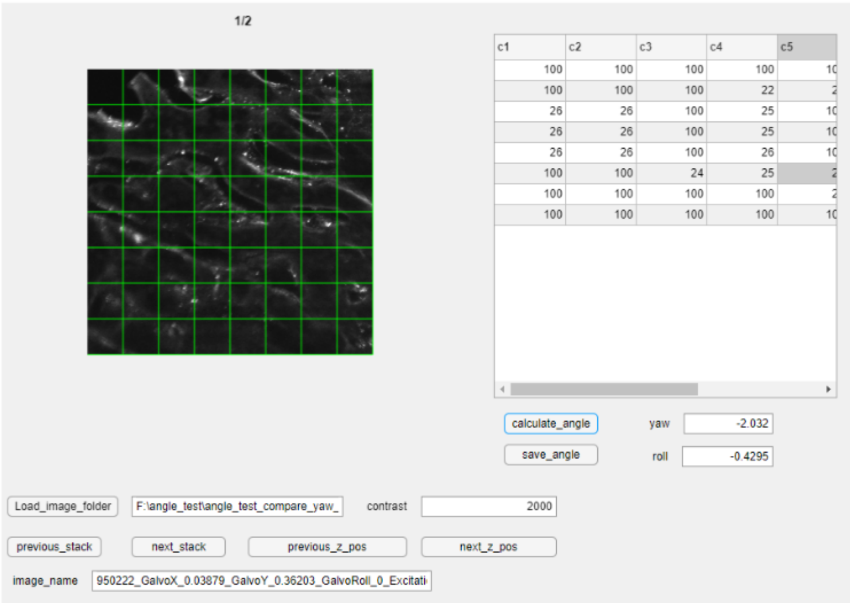

Fig. S2. Angle labeling tool. A self-built angle measurement tool was built to facilitate the labeling of the yaw and roll angles. The input image stack had 51 images in total (from -50 µm to 50 µm with 2 µm step size). The input stack was divided into 8 by 8 patches. By moving the z position, we could find the best image quality for each patch and record the corresponding z position. Then, using the labeled z position, the yaw and roll angles were calculated for this stack. The labeling tool is implemented in MATLAB.

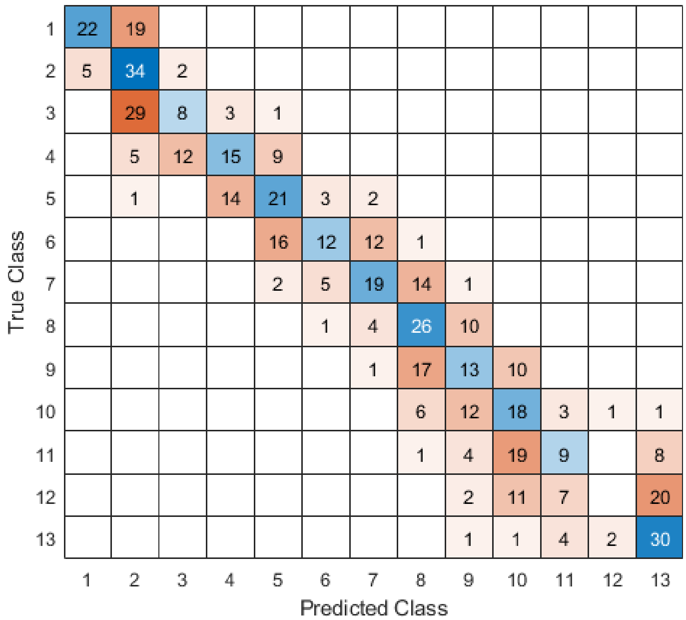

Fig. S3. The confusion matrix of the U-net model on the test dataset. The test set had 550 random test images in total. After removal of images that contained pure background 529 test images were kept. The correct predictions are on the matrix diagonal.

Table S1. Comparison between DCTS and U-net in terms of performance and speed.

	DCTS	U-net
Absolute distance error when performing autofocus on small patches (128 × 128 pixels)	8.56 µm	6.82 µm
Absolute distance error when performing autofocus on large patches (512 × 512 pixels)	5.86 µm	5.62 µm
Relative roll angle error	0.59°	0.53°
Relative yaw angle error	1.30°	0.63°
Image acquisition = stage movement plus integration time	13 images × 0.8 sec = ~10 sec	2 images × 0.8 sec = ~ 1.6 sec
Processing time (1024 × 1024 pixels)	For 64 patches (128 × 128 pixels each) = 2.56 sec	2.16 sec